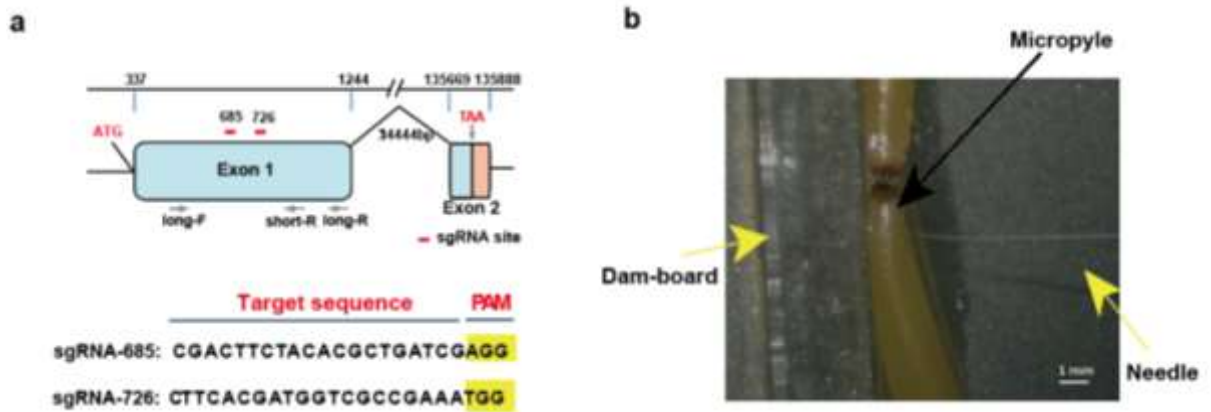


Egg tanning improve the efficiency of CRISPR/Cas9 mediated mutant locust by its enhanced defense ability after microinjection

Appendix A Structure of *LmdsRNase2* gene and micromanipulation of locust migratory eggs

a, there are two exons in the *LmdsRNase2* gene of migratory locusts. Two target sites of CRISPR/Cas9 were designed at the first exon by online design, and its corresponding sgRNAs were named as sgRNA-685 and sgRNA-726. b, microinjection operation. The microinjection site should be close to the head for higher editing efficiency.



Appendix B Primers used for synthetic of DNA template of sgRNA

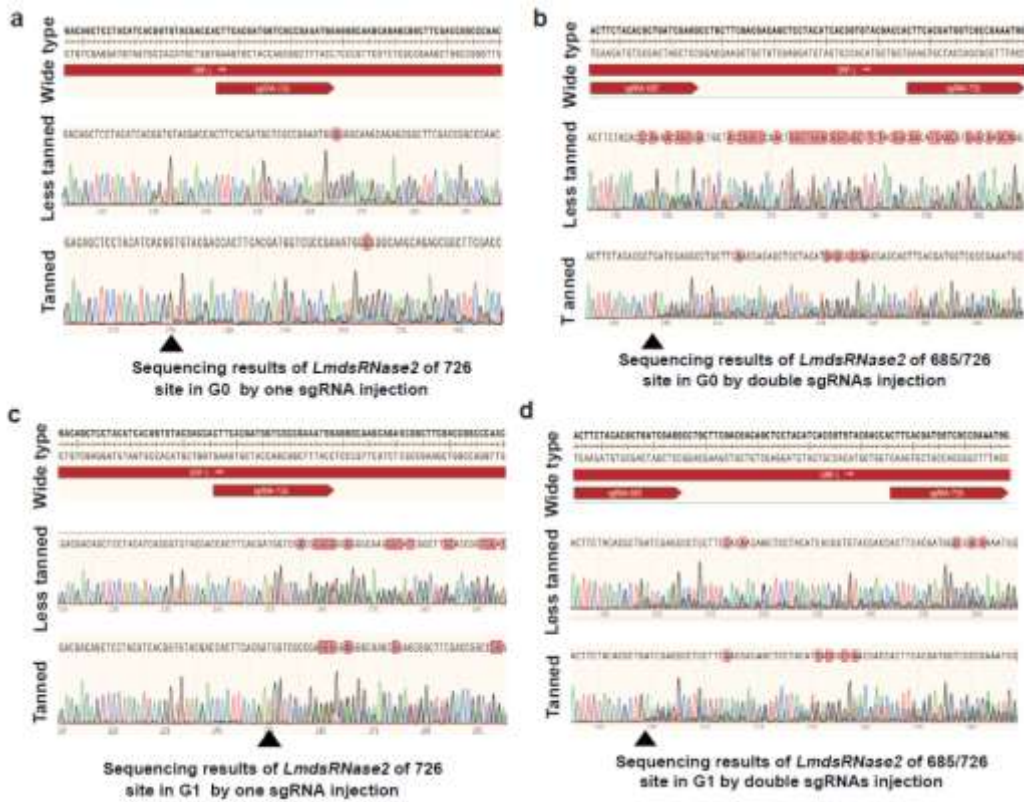
Name	Primer sequence (5'-3')
sgRNA-685-F	TAATACGACTCACTATAG GGAGCTGTCGTCGAAGCAGG
sgRNA-685-R	TTCTAGCTCTAAAAC CCTGCTTCGACGACAGCTCC
sgRNA-726-F	TAATACGACTCACTATAG CTTCACGATGGTCGCCGAAA
sgRNA-726-R	TTCTAGCTCTAAAAC TTTCGGCGACCATCGTGAAG

Appendix C Primers used for target gene sequencing

Primer name	Primer sequence (5'-3')
Long-F	CCTGTCCCGACAACAGTCTAC
Long-R	AAGTCGGATTTGGCAGTGAG
Short-R	GCTCGTTTGTACTGCTTGTC

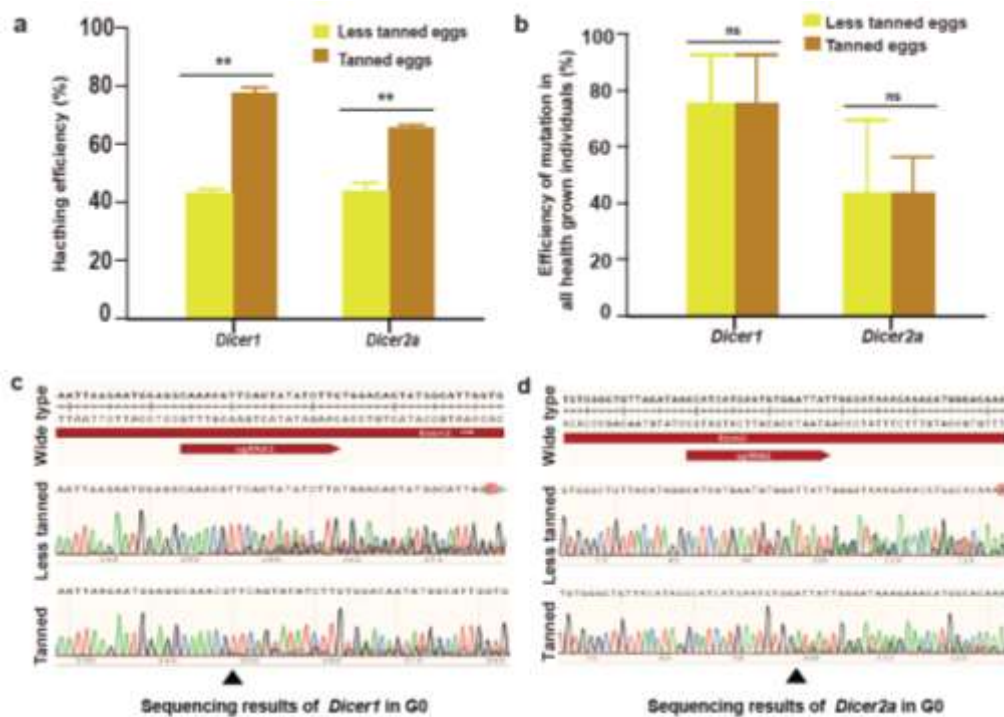
Appendix D Sequencing results of mutant individuals in G0 and G1 progeny

a-b, sequencing results of the G0 mutants in tanned and less tanned groups on target *LmdsRNase2*. c-d, sequencing results of the G1 mutants in tanned and less tanned group. The black triangle labeled the sites of double peak indicating the mutant sites caused by CRISPR/Cas9 editing.



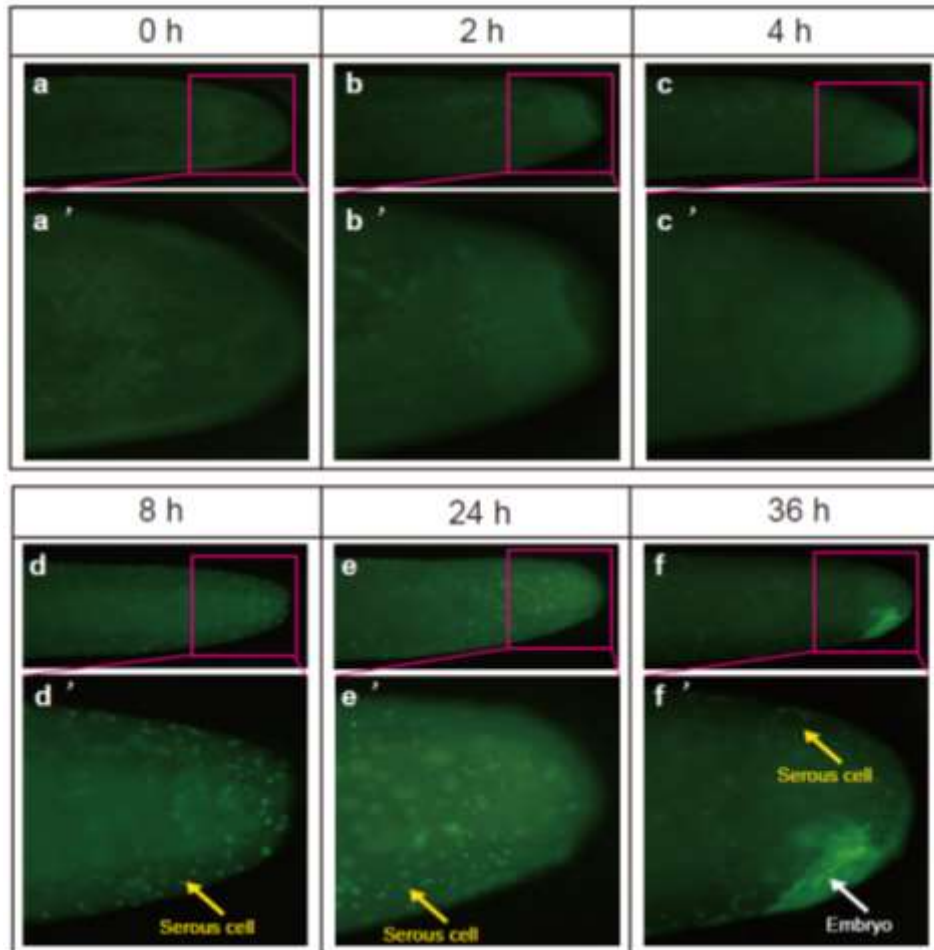
Appendix E Tanned eggs improved hatching rate of mutant individuals on different genes

a, comparison of the hatching efficiency between tanned and less tanned eggs injected with CRISPR/Cas9 system target on another genes *LmDicer1* and *LmDicer2a*. Each group contained 40 eggs and experiments were repeated three times. b, comparison of gene editing efficiency in health developed alive adults in G0 animals between less tanned and tanned eggs on *LmDicer1* (N=3) and *LmDicer2a* (N=4). Each group was repeated three times. c-d, sequencing results of the G0 mutants in tanned and less tanned group on *LmDicer1* and *LmDicer2a*. The black triangle labeled the sites of double peak, indicating the mutant sites caused by CRISPR/Cas9 editing.



Appendix F Staining of migratory locust egg nucleus

a-f, the SYTOX® stained the nucleus to green. The staining of the migratory locust eggs at 0 hour (a-a'), 2 hours (b-b'), 4 hours (c-c'), 8 hours (d-d'), 16 hours (e-e') and 32 hours (f-f'), indicated that new single green cells around the egg emerged after 4 h.



Appendix G The shell structure of insufficient tanned eggs and tanned eggs

a-c, yellow insufficient tanned eggshell structure describing the results in Figure 5. d-f, brown tanned eggshell structure describing the results in Figure 5.

